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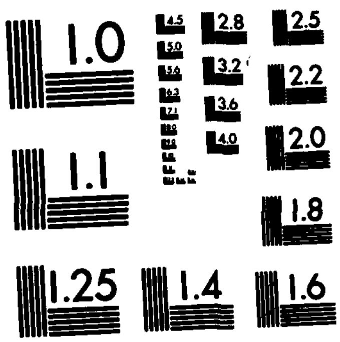


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REPORT TWO

# The Control of Dormancy and Development in *Coquillettidia perturbans* (Diptera: Culicidae).

Final Report

L. P. Lounibos

January, 1982

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD 17-79-C-9093

Florida Medical Entomology Laboratory

University of Florida

Vero Beach, Florida 32960



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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Biological information relevant to the control of Coquillettidia perturbans, a serious pest mosquito and disease transmitter, is provided. In south Florida larvae overwintered in the third and fourth larval stages. Between March and November two overlapping generations of adults were produced. Larval densities on cattails were correlated with host plant size, water temperature, and average stage of larval samples. Emergence traps conveniently and accurately measure adult densities. The related Mansonia dyari and Mansonia titillans

20. produced three generations annually from water lettuce, and weekly numbers of adults were correlated with water temperature. Laboratory colonization of C. perturbans was not achieved, but short-term maintenance of larvae on wheat roots permitted certain experiments. The intensity of larval diapause weakened between November and March. M. dyari attached more firmly to roots than did C. perturbans. The results reported here are interpreted in light of their relevance for Mansonia control, and avenues for future research are suggested.

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## SUMMARY

The current report describes the results of a two-year study on the control of development and dormancy of the mosquito Coquillettidia perturbans, a major pest and probable disease transmitter. The purpose of the research was to provide information pertinent to control of this mosquito as well as of related species which are important vectors of human filariasis and viruses in the tropics.

An abandoned phosphate pit, a major habitat of C. perturbans in south Florida, was studied in detail. Dormancy at this site occurred in both the third and fourth larval instars, and local populations of this species overwintered almost exclusively in these stages from mid-November through mid-March. Emergences of adult C. perturbans were detected as early as mid-March, but females of this species were not recorded until mid-April. Two, overlapping generations of adults emerged in 1981, peaks in abundance occurring in mid-June and early September.

Techniques improvised and used for sampling the egg, larval, and adult stages of C. perturbans are described. The emergence traps used in this study provided the most effective and sensitive method of monitoring sources of this species.

Larvae of C. perturbans were extracted from cattail plants growing in the former phosphate pit. In general, more larvae per plant were collected in the autumn and winter because larvae detach less readily in colder temperatures. Larval densities were negatively correlated with water temperatures but positively correlated with average instar numbers of samples. Significantly more larvae were recovered from larger cattails. No differences in larval densities were detected among samples near versus far from shore.

A canal clogged with water lettuce, habitat for three species of Mansonia including C. perturbans, was studied for one year in south Florida. Mansonia dyari and M. titillans were more abundant than C. perturbans, and the former two were multivoltine during the study period. M. dyari accounted for 89.7% of all mosquitoes captured in emergence traps, and numbers emerging per week were positively correlated with water temperature.

Attempts were made to culture C. perturbans larvae on wheat roots to assess the influence of photoperiod, temperature and food on larval dormancy. However, no modifications of rearing technique provided satisfactory cultures of this species and, thus, laboratory experiments were inconclusive in defining the importance of environmental stimuli for larval dormancy. Evidence from field collections indicated seasonal changes in the physiological status of overwintering fourth instar larvae.

In the laboratory, M. dyari larvae attached more firmly to roots than did C. perturbans.

Results obtained and reported herein are applicable to the control of C. perturbans. Four avenues suggested for future research include: (1)

improvement of sampling methods, (2) experimental manipulation of small habitat plots, (3) laboratory colonization, and (4) changes in human land use practices which lead to the creation of habitats suitable for this mosquito.

## FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals", prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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## I. Background

Mosquitoes of the genus Mansonia (s.l.) are important transmitters of human filariae and viruses in the tropics. The immature stages of this group are resistant to conventional control techniques because of their attachment to aquatic plants. The common North American species Coquillettidia perturbans is a serious pest in the eastern U.S. and is probably involved in transmitting certain viruses to man. Satisfactory control measures have not been developed for this species.

Although it has been known that C. perturbans overwinters in its larval stages, little information is available about the factors that control its dormancy and development. Data on natural populations have been limited by difficulties in sampling. Observations in the laboratory have been restricted by obstacles to rearing.

The purpose of the present study was to examine dormancy and development of C. perturbans with the objective of applying the acquired biological information to existing and future control programs. The study has investigated the seasonality of occurrence and relative abundance of growth stages of natural populations of this and related species in south Florida. New and improved sampling techniques allowed quantitative measurements heretofore unavailable. Laboratory cultures permitted certain observations under controlled conditions.

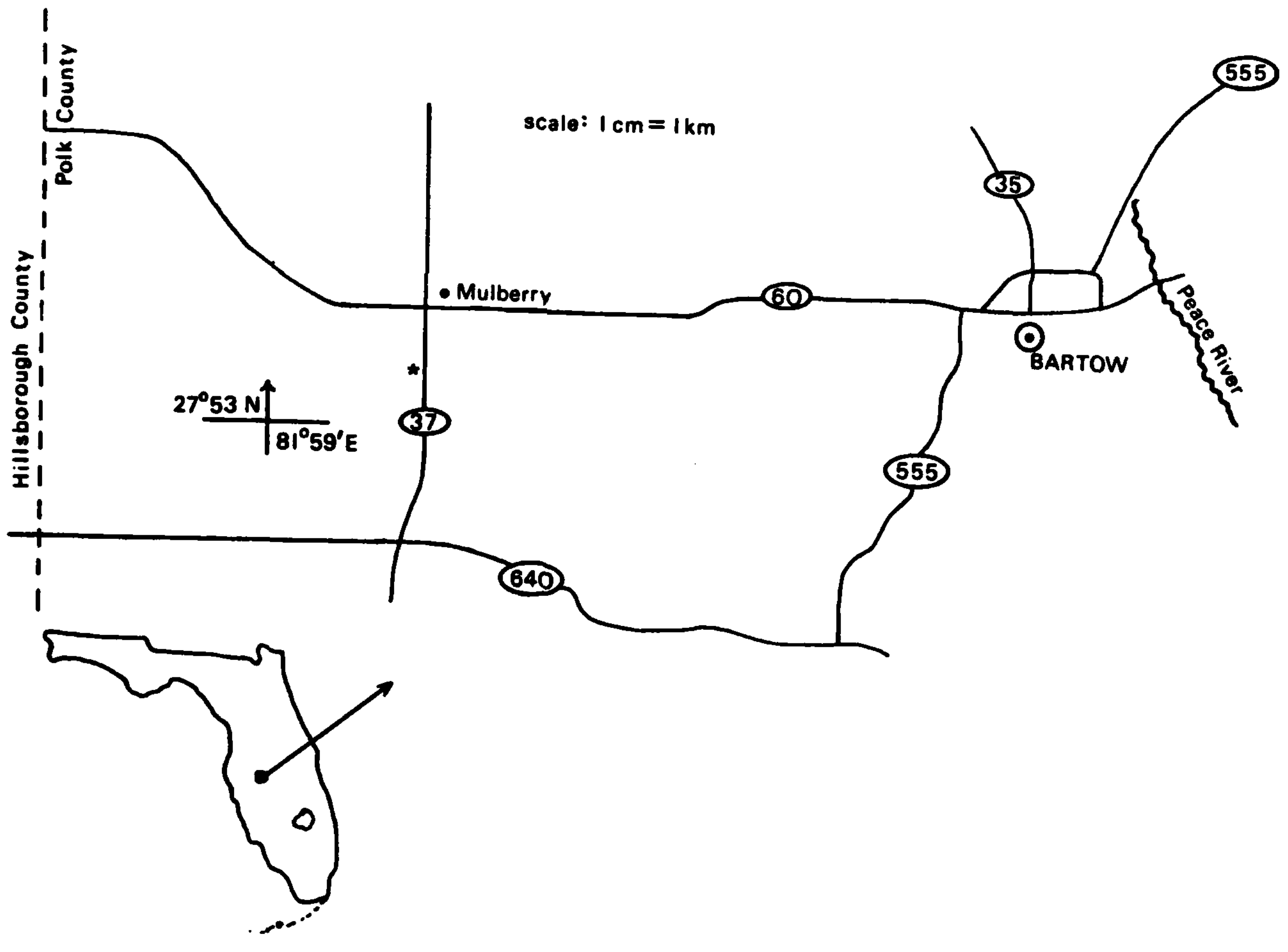
## II. Studies on a natural population of Coquillettidia perturbans at Mulberry, Florida.

### Site description

Previous (unpublished) reports by Polk County Environmental Services had indicated that abandoned phosphate mines were important sources of Mansonia mosquitoes in that county. We chose to study a single, abandoned pit of 17.2 acres located south of Mulberry (Fig. 1). This particular site was chosen because (a) of its accessibility, (b) it was representative of older, abandoned mines in the area, and (c) it was covered by a (floating) mat of vegetation (Fig. 2) known to support Mansonia mosquitoes, especially C. perturbans. Further, the proximity of the site to Polk County Environmental Services facilitated collaboration, essential to our sampling program, between our staff and theirs.

The matrix of vegetation and organic material covering the pit at Mulberry varied in thickness from ca 5-50 cm depending on location and season. The mat was generally thinner in the colder, winter months. Three species of aquatic plants dominated the total vegetative biomass: Eichornia crassipes (Mart.) Solms (water hyacinth), Typha sp. (cattail) and Ludwigia octovalis (Jacq.) Raven (primrose willow). Water hyacinth was clumped in areas where the mat was thin and water was exposed. By contrast, cattails and primrose willows became established only where the organic layer was thick enough to support their roots. Other species of aquatic and semi-aquatic plants co-occurring in the abandoned pit included: Lemna minor L. (duckweed), Azolla sp. (water-fern), Salvinia rotundifolia Willd., Ipomea aquatica Forsh (morning glory), Limnobium spongia (Bosc.) Steud. (frog's bit), Hydrocotyle umbellata L. (water pennywort), Pontederia sp. (pickerelweed), Utricularia sp. (bladderwort), Habernaria sp. (orchid) and undetermined sedges and grasses.

Fig. 1. Map of west-central Polk County, indicating the location (asterisk) of the abandoned phosphate mine in Mulberry used as a study site for Coquillettidia perturbans.



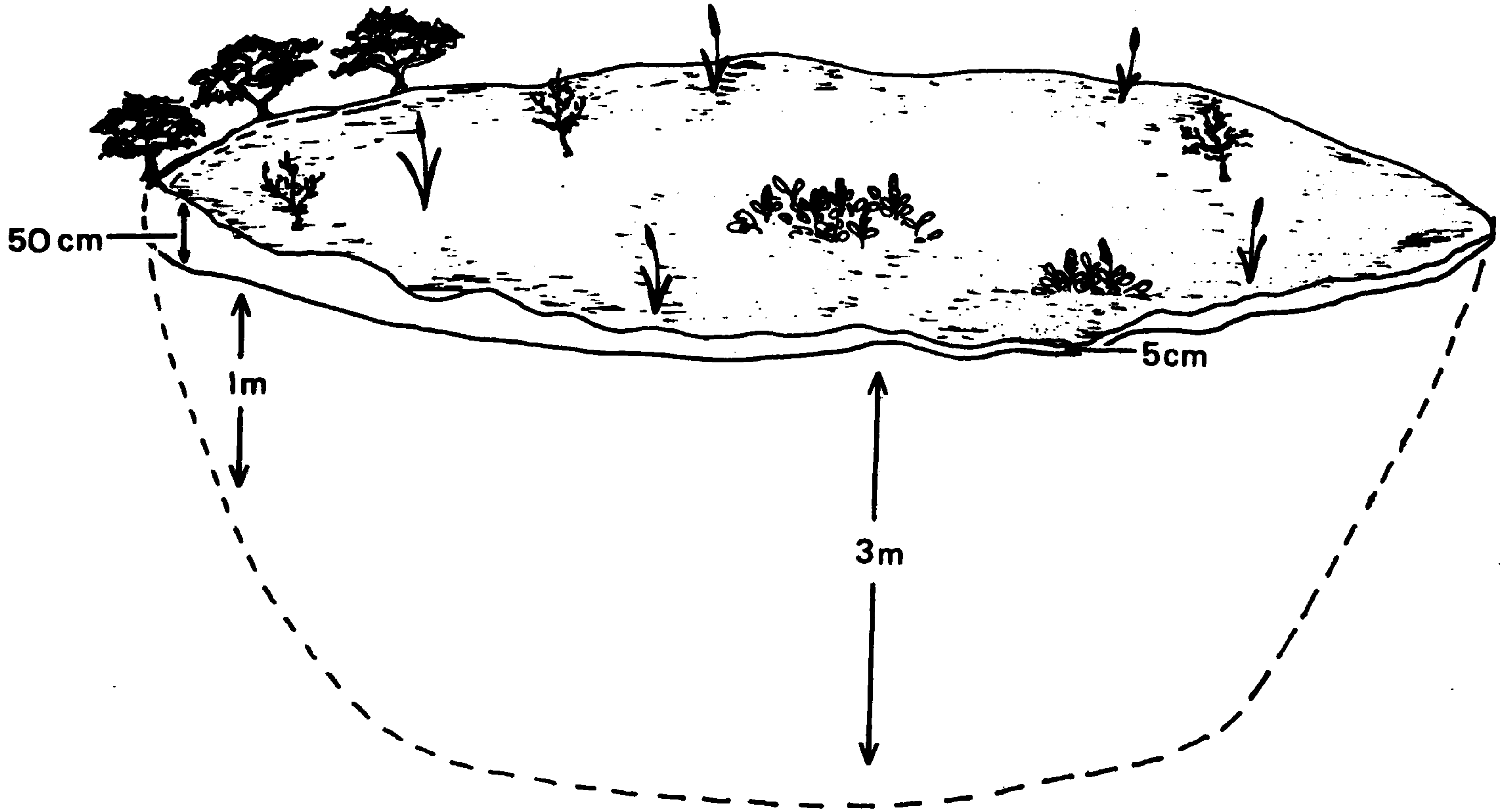



Fig. 2. Schematic representation of the floating mat at Mulberry. Mat thickness (5-50 cm) and water depth (1-3 m) are not drawn to scale.

Water depth beneath the floating mat ranged from ca 1 m near shore to ca 3 m near the center. Since the mat was too thick to permit transport by boat, yet too thin to support a man on foot, movement within the habitat was achieved on styrofoam "skis". These skis distributed an individual's weight and provided sufficient flotation for safe passage on top of the mat.

At the study site rainfall and temperature at the level of plant roots were continuously monitored on recording instruments (Weathermeasure; Tempscribe) mounted on an old surfboard situated ca 30 m from the shore. The instruments were checked and charts changed weekly, at which time dissolved oxygen (DO) was measured (Yellow Springs Instruments) at two points near shore where the mat was loose. The mean weekly DO reading during twenty months of measurements was $0.38 \pm 0.28$ (SE) ppm (n=130). On only five occasions were values greater than 1.0 ppm recorded.

## Larvae

### sampling procedure

Preliminary investigations revealed that *C. perturbans* larvae were attached to the roots of most aquatic plants found in the floating mat. We chose to sample only cattails (*Typha* sp.) because of their abundance and relative ease of extraction. Medium-sized (1-2 m above-ground height) cattails were uprooted and placed into buckets of ca 20 l capacity. Plants with damaged roots were discarded. On shore, shoots were cut off and discarded and water added to the holding buckets for transport of roots to the laboratory.

At Vero Beach the contents of buckets were poured into garbage pails of ca 88 l capacity. To dislodge attached larvae, each clump of cattail roots was shaken vigorously five times in tap water added to the pails; roots were subsequently discarded. An insert, originally part of a trap for collecting *Mansonia* larvae in nature[1], was fitted snugly into the garbage pail, and tap water was added to a level a few cm above the cone-shaped aperture of the insert. Larvae deprived of roots for attachment, surfaced through the cone-shaped apertures, but were prevented from submerging to the bottom of the pail by the construction of the insert (see[1]). After twenty-four hours of exposure, the surface water in the insert was siphoned off, and larvae were counted and separated by instar in the laboratory.

On seven occasions in 1980 the inserts were set over field samples for a second twenty-four hour period to measure the efficiency of the extraction procedure. The number of larvae recorded in the second extraction period amounted to from 8.3% to 29.2% ($\bar{x}$ = 16.0%) of the original sample.

Other problems with the sampling and extraction procedure remain and are mentioned here as *caveats* in data interpretation. An unknown number of larvae detached from roots when cattails were pulled from the floating mat; an effort to measure larval detachment after disturbances in laboratory aquaria is described in section III of this report. Predators, especially fish, sometimes co-occurred with and likely reduced the numbers of larvae in garbage pails. Finally, the pre-imaginal stages attached to roots were differentially sampled. Specifically, pupae and first instar larvae were

underrepresented in our collections proportionate to their expected frequencies of occurrence in nature.

larval phenology

Between July 1978 and December 1980 3144 cattails were pulled and extracted for larvae in the course of 36 visits to Mulberry at approximately four-week intervals. An average of 87.3 $\pm$ 21.9 (SE) cattails were collected per visit, and a total of 11,043 larvae and pupae of C. perturbans were identified and staged.

The larval stages of C. perturbans showed definite seasonal progressions in relative abundance which were consistent between years. Third and fourth instars accounted for 98.7 to 100% of all larvae between January and April of both 1979 and 1980 (Fig. 3). Fourth instars comprised over 90% of all individuals during this period in 1980 and over 65% of specimens during January - April 1979. In May of both years, first and second instar larvae increased as the proportions of fourth instars decreased.

In June and July of 1979, second instars were the most abundant stage recovered; a peak in the abundance of this stage also occurred in July of 1978 and 1980. In the warm summer months these second instars apparently molted promptly to the third stage, which was the commonest larval stadium observed in all collections between August and October in all three years. However, by early November of all years, the proportions of instars had shifted towards a predominance of fourths.

Although first instar larvae and pupae are believed to be underrepresented in collections, in both 1979 and 1980 the maximum number of pupae were recorded in late April or early May, and the maximum number of first instar larvae in late May or June. First instar larvae were recovered from samples as late as November in 1978 and 1980.

larval densities

Estimates of the number of larvae on a cattail plant were calculated by dividing the number recovered from a garbage pail by the number of roots (usually ten) shaken in the pail. The range in numbers of larvae per cattail was large, both among samples on a collection date and between dates (Fig. 4). However, there was some evidence of seasonal consistencies in larval densities. Namely, fewest larvae per plant were recovered during the summer between July and September, and densities generally increased in the period between October and December.

A seasonal pattern of larval densities was less evident in the first half of either 1979 or 1980 (Fig. 4). A peak in mean numbers per plant occurred in June of 1979 when all larval stages were present (Fig. 3), but the greatest density of 1980 was recorded in April when only pupae (0.5%), fourth (91.2%), and third (8.3%) instars were encountered. Seemingly, local patchiness in occurrence (clumping) of C. perturbans larvae may obscure seasonal trends in density.

Fig. 3. The seasonality of instars of C. perturbans at the abandoned phosphate pit in Mulberry. All collections except the first four in 1978 represent counts of > 100 larvae ($\bar{x}$ = 306.0 ± 227.5 (SE), n = 36).

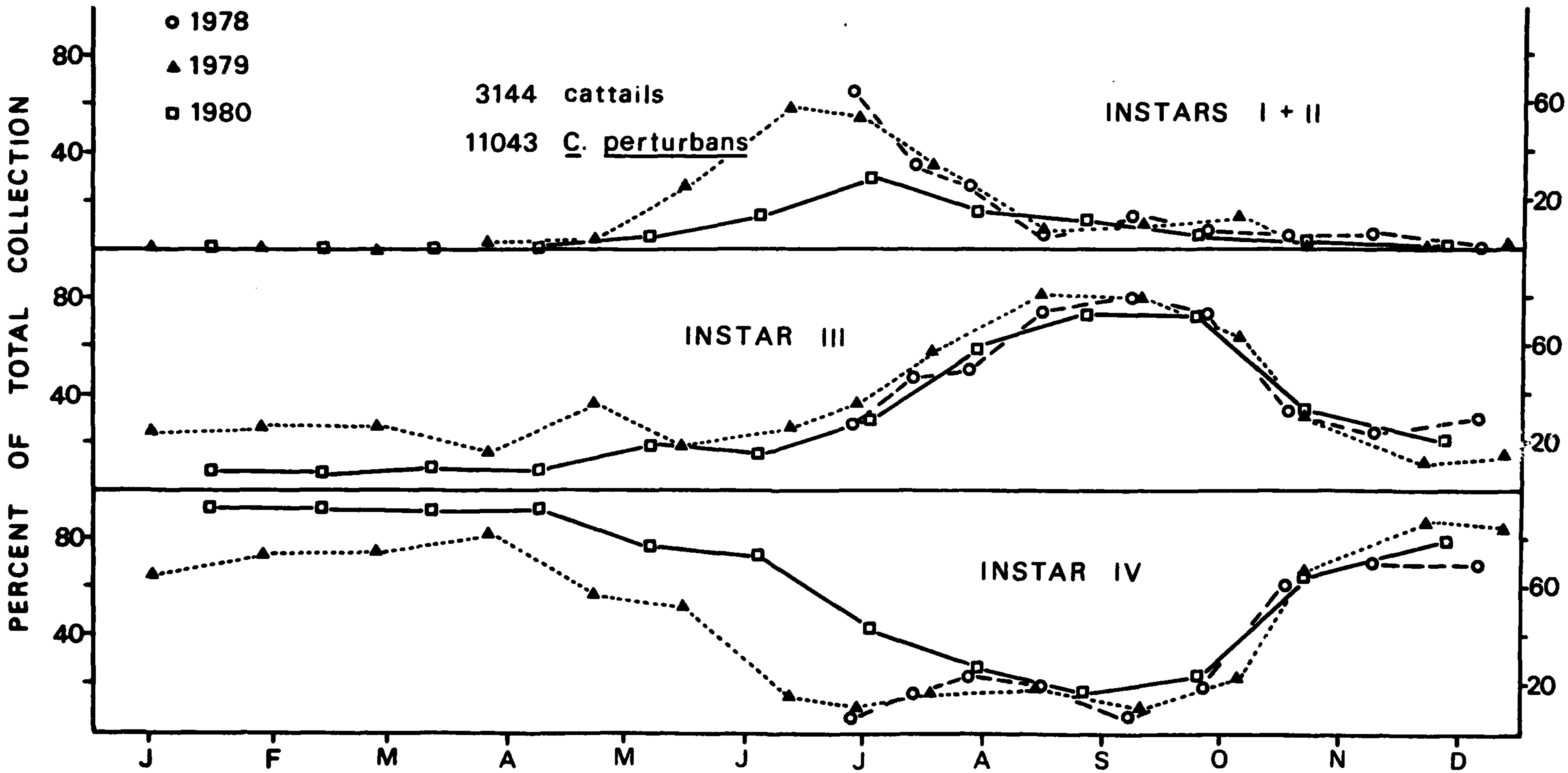
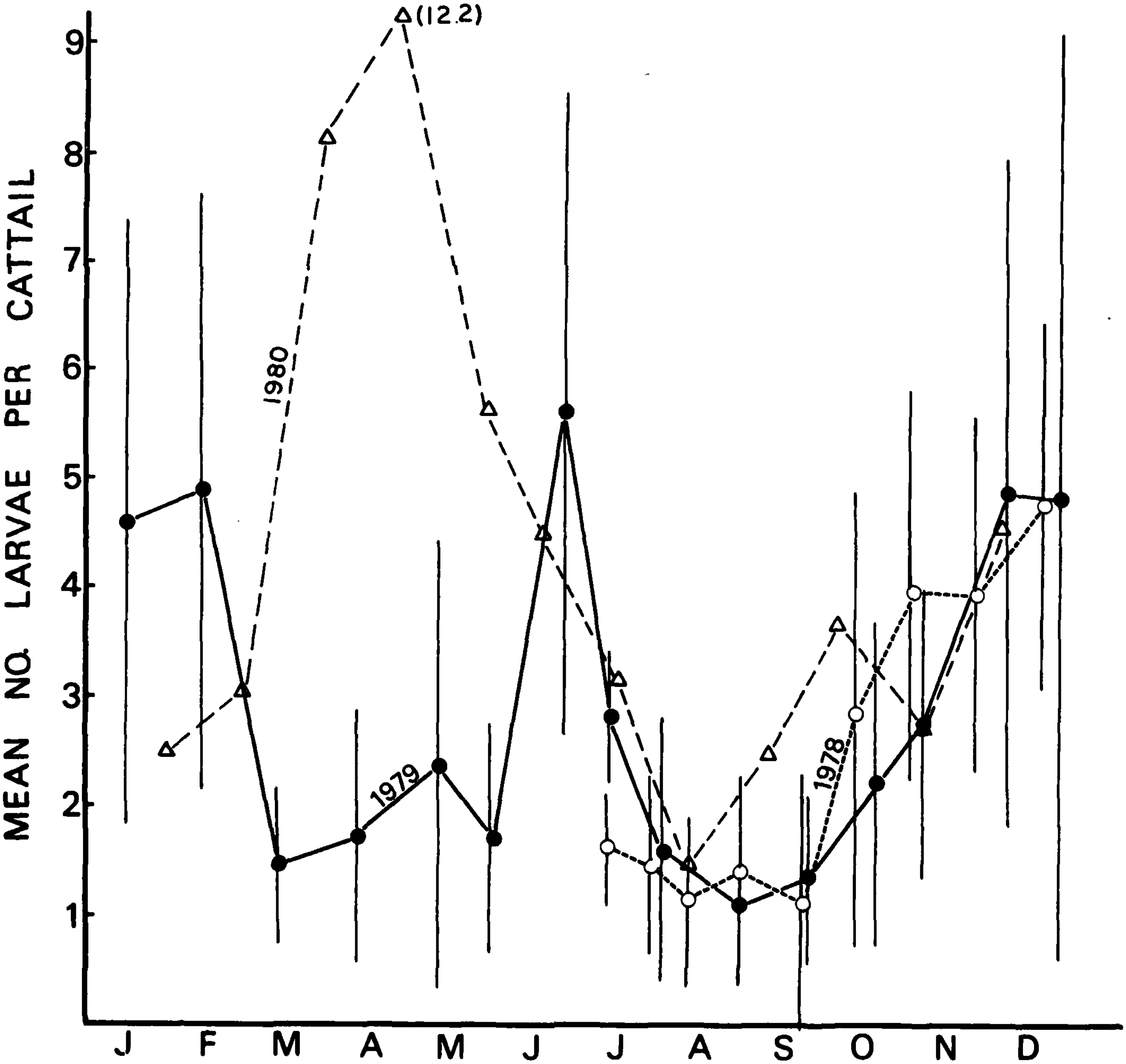


Fig. 4. Mean densities of C. perturbans per cattail root from 36 sampling occasions during 1978-80. Vertical bars represent $\pm$ 1 SE, omitted for clarity from the 1980 data.



Larval densities per ten cattails were positively correlated with the average instar number of a sample (Fig. 5). In effect, more larvae were recovered per plant when older instars predominated in samples. Since average instar number increased in the colder months when larvae diapaused in the later instars, there was a significant negative correlation between larval density and water temperature at the time of sampling (Fig. 6).

On five sampling occasions between November 1980 and January 1981, we explored the relationship between cattail size, measured as dry weight of roots, and number of C. perturbans recovered. This analysis was conducted in the autumnal and winter period when the larval population was primarily in the third and fourth instars. The average instar numbers of samples from individual plants did not differ significantly between collection dates ($F_{4,16} = 1.8$, $P > 0.1$). Cattail roots were extracted as described previously, except each plant was individually isolated, and after shaking to dislodge larvae, roots were dried in an oven at 80°C.

Root dry weight ranged from 2.3 - 38.1 gm ($\bar{x} = 14.5$) and from 0 - 38 larvae (median = 6.5) were recovered from twenty-five plant samples (Fig. 7). Root weight and C. perturbans abundance were positively correlated ($r_s = 0.48$, $P < 0.05$) i.e., larger cattails may be expected to harbor more larvae.

## larval distribution

Between July 1978 and March 1979 we sorted cattail samples based on their proximity to shoreline at the Mulberry pit. The mean number of larvae per cattail recovered near (< 13 m distant from) shore was 2.91 ± 1.68 (SE) which was not significantly different from samples more distant (≥ 33 m) ($\bar{x} = 2.84 \pm 1.73$) for eleven sample dates ($t_s = 0.10$, $P > 0.9$, 20 df). Thus, at least for the small area of habitat here sampled, C. perturbans were neither more nor less abundant near or away from shore.

## Egg rafts

Owing to the success of Canadian investigators[2] in recovering the free-floating egg rafts of C. perturbans within circles of plastic tubing set on water surfaces expected to attract oviposition, five plastic hoops of ca 1 m diameter were set upon the floating mat to define an area for egg sampling. Most plants within the confines of the circles were uprooted to expose open water, but a few plant stalks, expected to attract gravid females, were allowed to remain. Weekly, beginning in early February 1980, the water surfaces confined by the hoops were skimmed with a dipper containing a fine-mesh bottom, and the contents of these collections returned to the laboratory for inspection. No egg rafts were ever recovered, and the weekly sampling was discontinued in September 1980. Larvae of Anopheles sp. and Uranotaenia sp. were routinely collected in the dipping samples.

Fig. 5. The relationship between C. perturbans density and the average instar number (AIN) of samples from Mulberry. AIN = $\frac{\Sigma I(n_i)}{n_t}$ where

$n_i$ = the number of individuals in an instar, I = instar number (1-4), and $n_t$ = total number of individuals. Data are from all samples between July 1978 and December 1980. r is the product-moment correlation coefficient.

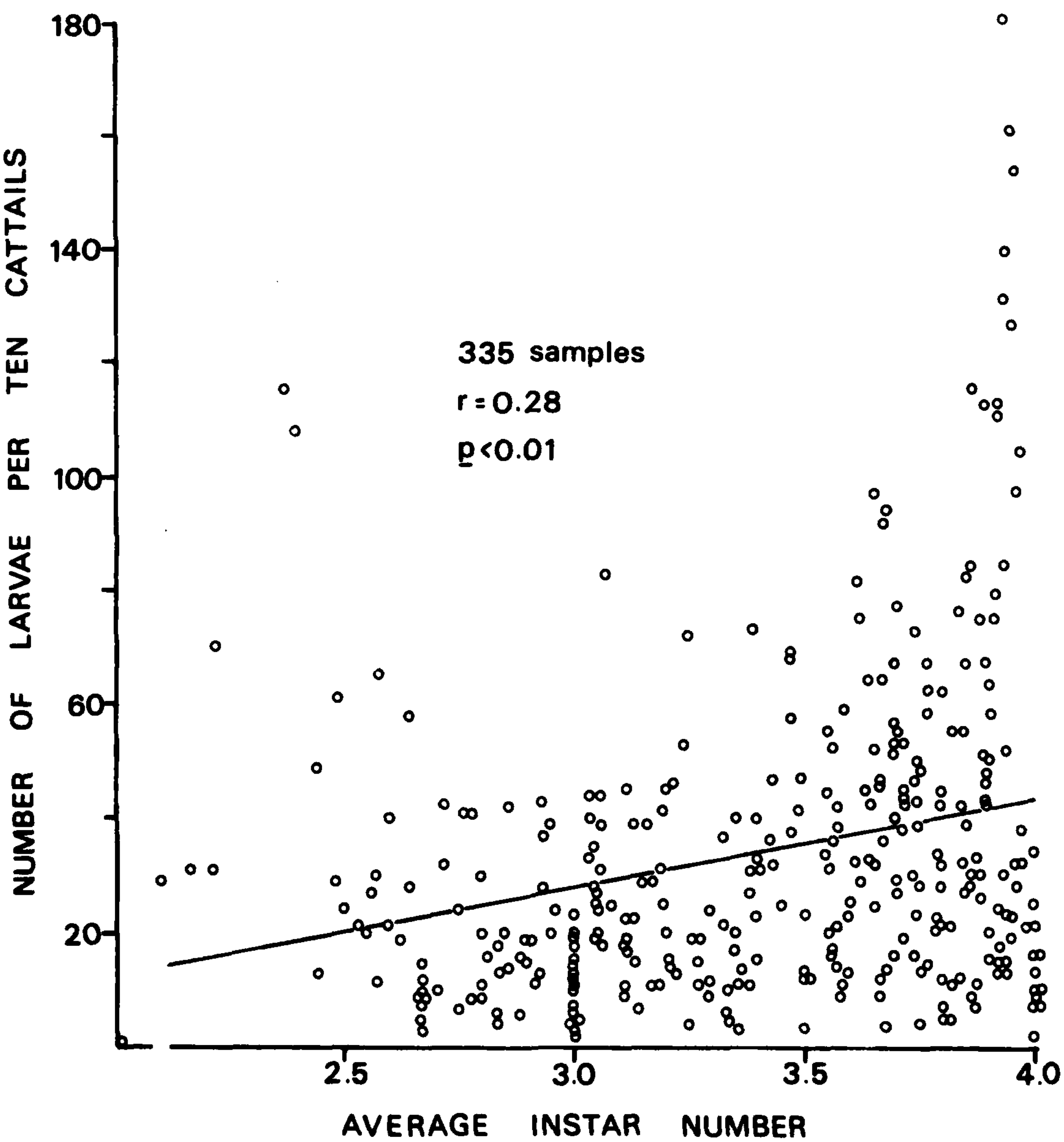


Fig. 6. The relationship between larval density of C. perturbans and water temperature at the level of cattail roots recorded on that collection date. r is the product-moment correlation coefficient.

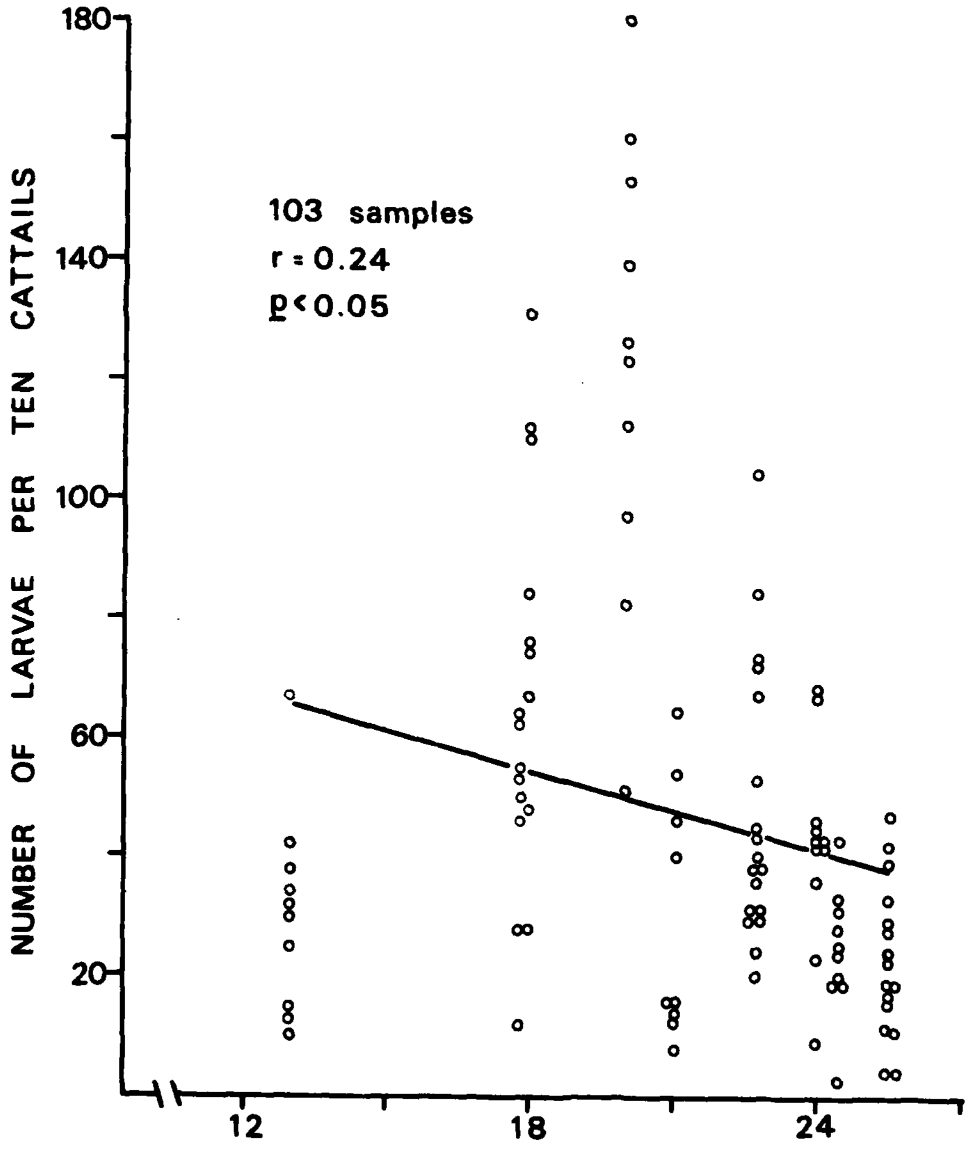
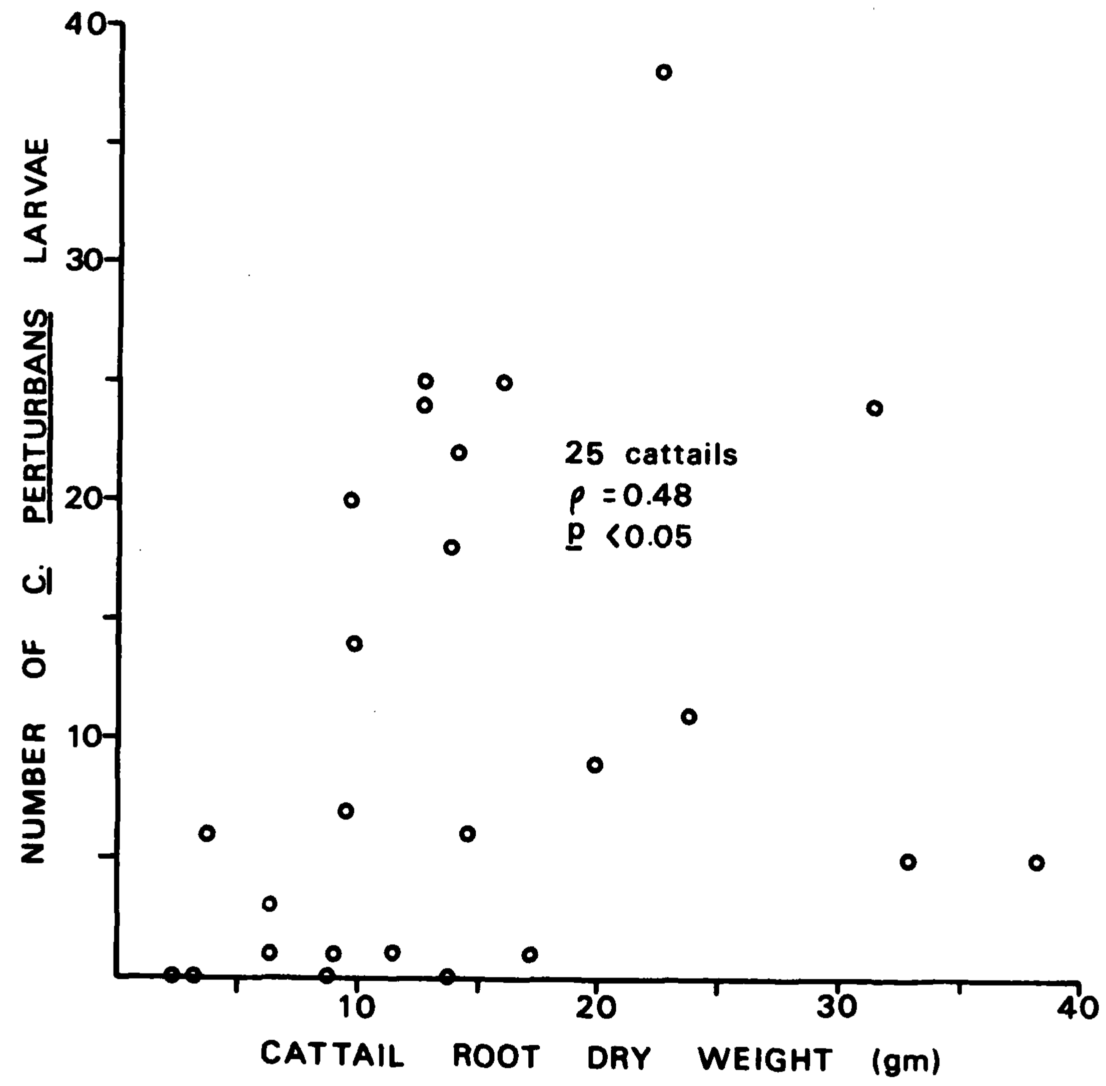


Fig. 7. The relationship between the number of larvae extracted from 25 cattails and root dry weight. ρ (rho) is Spearman's (rank-order) correlation coefficient.



## Adults

### light trap

A CDC light trap was operated 100 m from the Mulberry pit one night per week beginning February 1980. As the trap duplicated sampling efforts being conducted by Polk County Environmental Services, its continued maintenance by this project was not justified and it was discontinued in September 1980. The five commonest mosquito species captured between February and September and their rank order of abundance were: C. nigripalpus > C. perturbans > M. titillans > C. salinarius > A. quadrimaculatus.

### emergence traps

Preliminary data and reports by other researchers[2,3] suggested that emergence traps might provide relatively unbiased information on the seasonality of C. perturbans adults. Three pyramids encompassing 4 $m^2$ and ca 1 m high were constructed of wooden or aluminum frames and covered with nylon screen. The original concentrating devices consisted of removable screen cylinders, ca 4.0 cm in diameter and 15 cm long, affixed on top of the pyramid; these were changed and examined at weekly intervals beginning in February 1980. However, as temperatures rose predators, especially ants, increased, whereupon numbers of adult C. perturbans decreased in traps (Fig. 8), suggesting that adult mosquitoes were being eaten in emergence traps. In September 1980 the concentrating devices were redesigned to correspond to Model WEEK of LeSage and Harrison[4] such that flying insects would fall into a replaceable bottle containing a liquid preservative. Redesigned traps were in place between October 1980 and October 1981, during which period bottles were changed and the traps cleaned of predators (mainly spiders and ants) weekly. At 6-8 week intervals during periods of C. perturbans emergence, traps were repositioned on the floating mat to compensate for the possible depletion of the mosquito subpopulation under the trap.

In both 1980 and 1981, no adult C. perturbans emerged between January and mid-March (Fig. 8). The first adults, exclusively males (Figs. 9 & 10), appeared in the latter half of March in both years (Fig. 8). In 1980 the numbers of C. perturbans increased in weekly collections until mid-May and thereafter delined; this decline was more likely attributable to the inefficiency of the collection device rather than an actual decrease in numbers of C. perturbans emerging.

In 1981 C. perturbans adults were recovered from one or more of the three traps in every weekly collection from mid-April through the end of October. The seasonal changes in overall abundance (Fig. 8), and the timing of occurrence of males and females (Fig. 9 & 10), suggested that two, overlapping generations emerged during this six-month interval. The first broad emergence period occurred from mid-April through mid-July. This was followed by a brief depression of adult numbers in traps and a subsequent recrudescence in early August which continued until mid-October. Maxima of more than 100 adults/week in the three emergence traps were recorded in mid-June and early September (Fig. 8). Individual traps, while varying considerably in absolute numbers captured (Table 1), showed similar trends in the seasonal abundance of C. perturbans (Fig. 9 & 10).

Fig. 8. The seasonal abundance of adult *C. perturbans* recovered between February 1980 and October 1981 in three (4 $m^2$ each) emergence traps at Mulberry. Circles signify collections when only two traps operated. During September and October 1980 traps were removed, redesigned (see text) and reinstated.

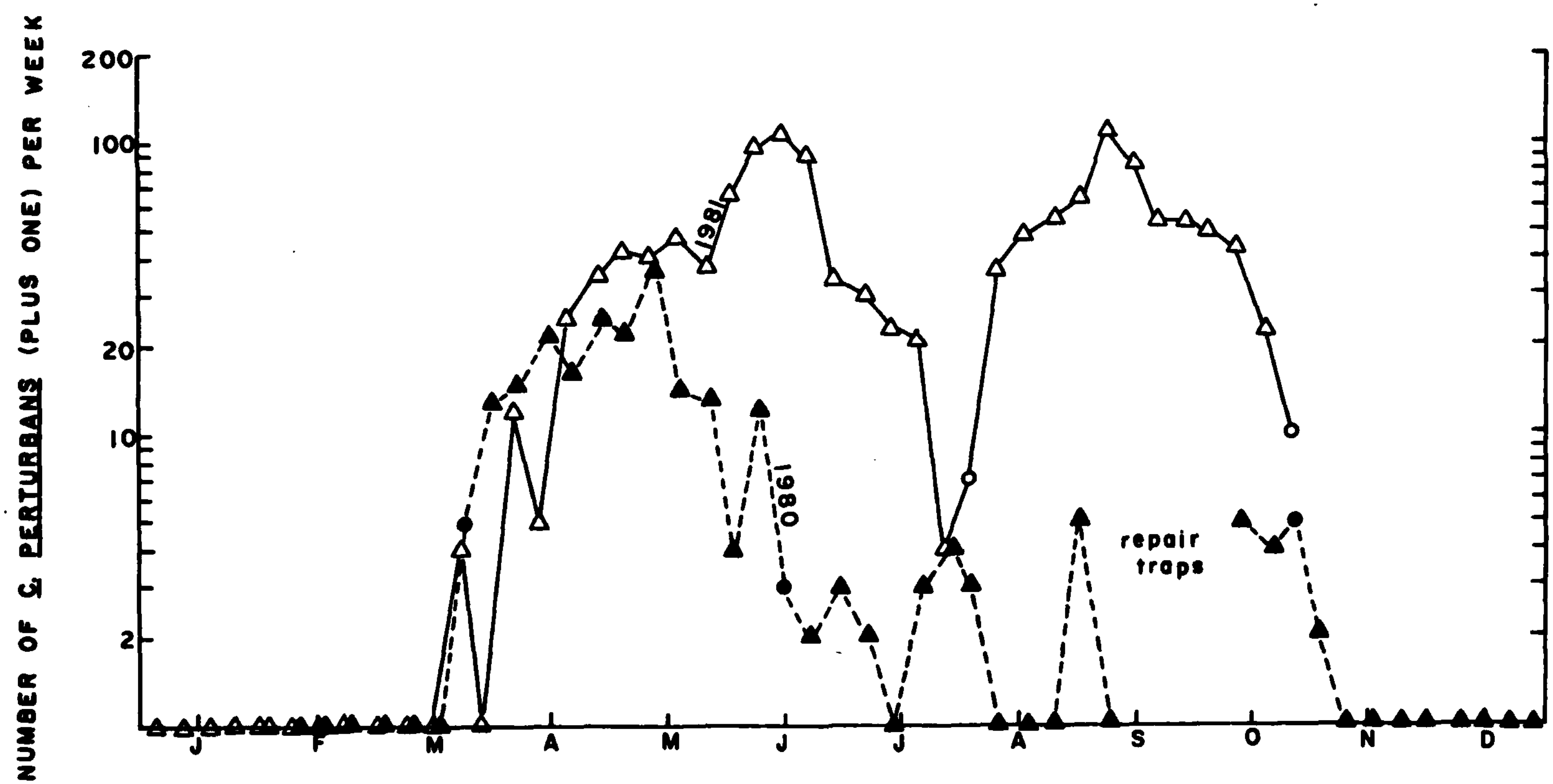


Fig. 9. The abundance of male (open circles) and female (closed circles) C. perturbans in trap number one at Mulberry during the emergence period of 1981. Arrows indicate repositioning of the trap. No data were collected in the week of July 29 - August 5.

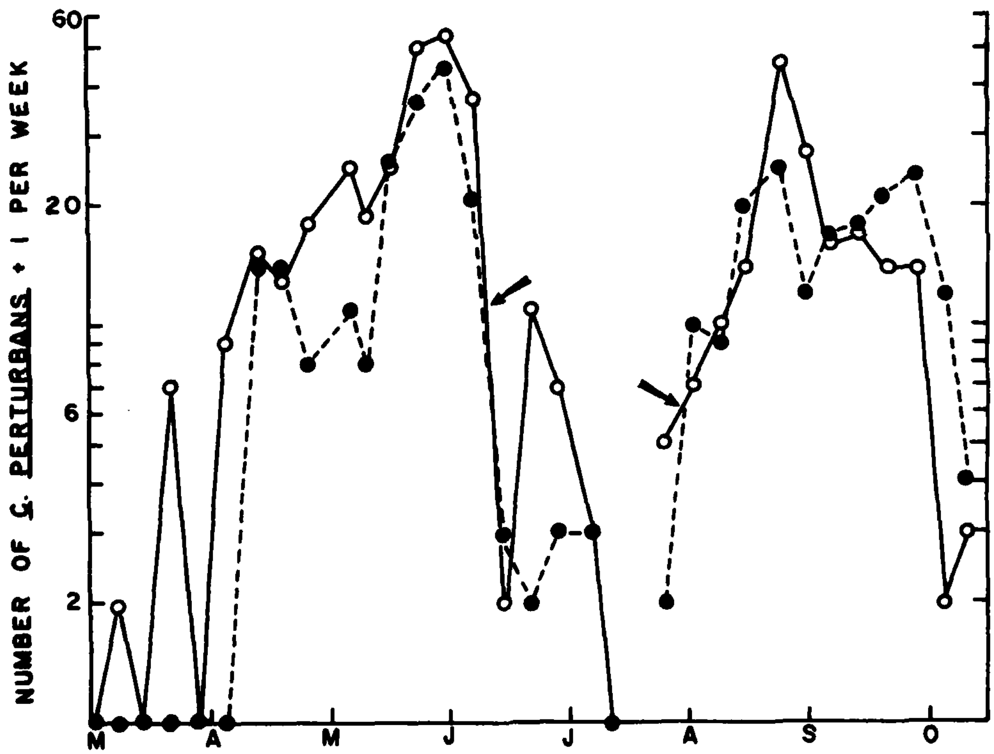


Fig. 10. The abundance of male (open circles) and female (closed circles) C. perturbans in trap number two at Mulberry during the emergence period of 1981. Arrows indicate repositioning of the trap.

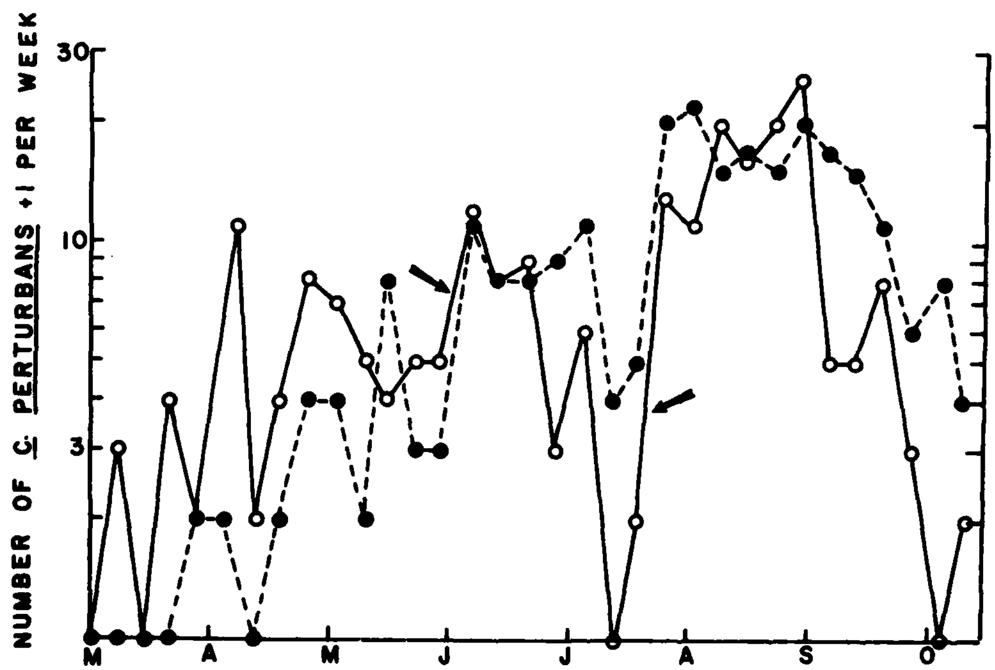


Table 1. Numbers of C. perturbans adults captured in emergence traps at Mulberry, Florida between October 1980 and October 1981.

| Trap No. | Males | Females | $X^2_{(1)}$ |
|---|---|---|---|
| 1 | 448 | 348 | 12.56*** |
| 2 | 200 | 229 | 1.96 ns |
| 3 | 86 | 29 | 28.25*** |
| | 734 | 606 | 12.23*** |

$H_o$ : sex ratio 1:1

*** = $P < 0.001$

ns = not significant

If the bimodal emergence pattern of 1981 (Fig. 8) represents the occurrence of two generations, two shifts in sex ratio, males preceding females in relative abundance, might be expected. As anticipated, male C. perturbans generally preceded females during the spring-summer emergence (Figs. 9 & 10). However, a similar male:female pattern in the purported summer-autumn generation is less clear, likely because of overlap with the preceding generation. If females from overwintering larvae were still emerging in August, their occurrence could disguise the expected precedence of males in the second generation. In both traps examined, females outnumbered males from late September onwards (Figs. 9 & 10), supporting the hypothesis of bivoltinism in this population. In two of the three traps, sex ratio during one complete annual cycle was male-biased (Table 1); at present this observation cannot be accounted for.

All mosquitoes captured in emergence traps were identified to species. Between October 1980 and October 1981, 1814 specimens were identified, and the five commonest (exclusive of C. perturbans) are tallied in Table 2. C. perturbans accounted for 73.9% of all specimens, followed by A. crucians, 17.5%, and C. nigripalpus, 6.2%.

## Discussion

The sequences of larval stages of C. perturbans encountered at Mulberry are in general agreement with the seasonal pattern observed by Bidlingmayer[5] at Leesburg slightly to the north. At Leesburg, however, larvae overwintered almost exclusively in the fourth stage, whereas at Mulberry 10-35% of the overwintering generation remained in the third instar.

Light trap records reported by Bidlingmayer[5] suggested the occurrence of two generations of C. perturbans at Leesburg, the larger recorded in May and June and a lesser one in September. This annual pattern of apparent bivoltinism conforms to the emergence data for 1981 from Mulberry, although

Table 2. Numbers of the five commonest mosquito species which co-occurred with C. perturbans in emergence traps at Mulberry October 1980 - October 1981

| Trap No. | A. crucians | C. nigripalpus | U. sappharina | C. erraticus | M. titillans |
|---|---|---|---|---|---|
| 1 | 218 | 96 | 16 | 0 | 3 |
| 2 | 58 | 4 | 8 | 8 | 1 |
| 3 | 42 | 12 | 3 | 0 | 0 |
| | 318 | 112 | 27 | 8 | 4 |

no differences in sizes of generations were observed at the latter site. Analyses of light trap records from Myakka River State Park led Provost[6] to conclude that C. perturbans experienced three annual generations in south Florida. Although a third generation at Mulberry is conceivable, if such occurs it is considerably smaller than the preceding ones. Mulberry, which is approximately equidistant between Leesburg and Myakka River on a north-south transect, may represent an intermediate zone in emergence patterns between the two sites.

The data from Mulberry provide quantitative information useful for the control of C. perturbans. The first emergence of adults in March is apparently well synchronized between years (Fig. 8), suggesting that this event may be locally predictable. Females were not recorded until approximately one month after males, recommending that adult control of this species might profitably be delayed until April. The complete shutdown of adult emergence from early November through mid-March means that the entire population of C. perturbans is confined to its aquatic habitat for the winter. If satisfactory control methods for the preimaginal stages could be developed, the pcpulation would be most sensitive to treatments at this time. The winter months are also the most profitable time for comparing larval populations among sites, since more larvae are recovered from host plants in the winter.

## III. Studies on natural populations of Mansonia mosquitoes at Indrio, Florida.

### Rationale and site description

During the course of investigations supported by this contract, a new field site was chosen. This locality possessed several properties which suited it for sampling and measuring Mansonia populations. Firstly, the site is located in NE St. Lucie County, ca 8 km from our Vero Beach laboratory (Fig. 11). The canal which supports Mansonia immatures is hidden from public view, and the owner is amenable to our use of his property. Secondly, the canal is overgrown with a single species of aquatic plant, Pistia stratiotes L., which can be identified with fair assurance as the host for attachment of most Mansonia. P. stratiotes is a simpler subject for larval-plant studies

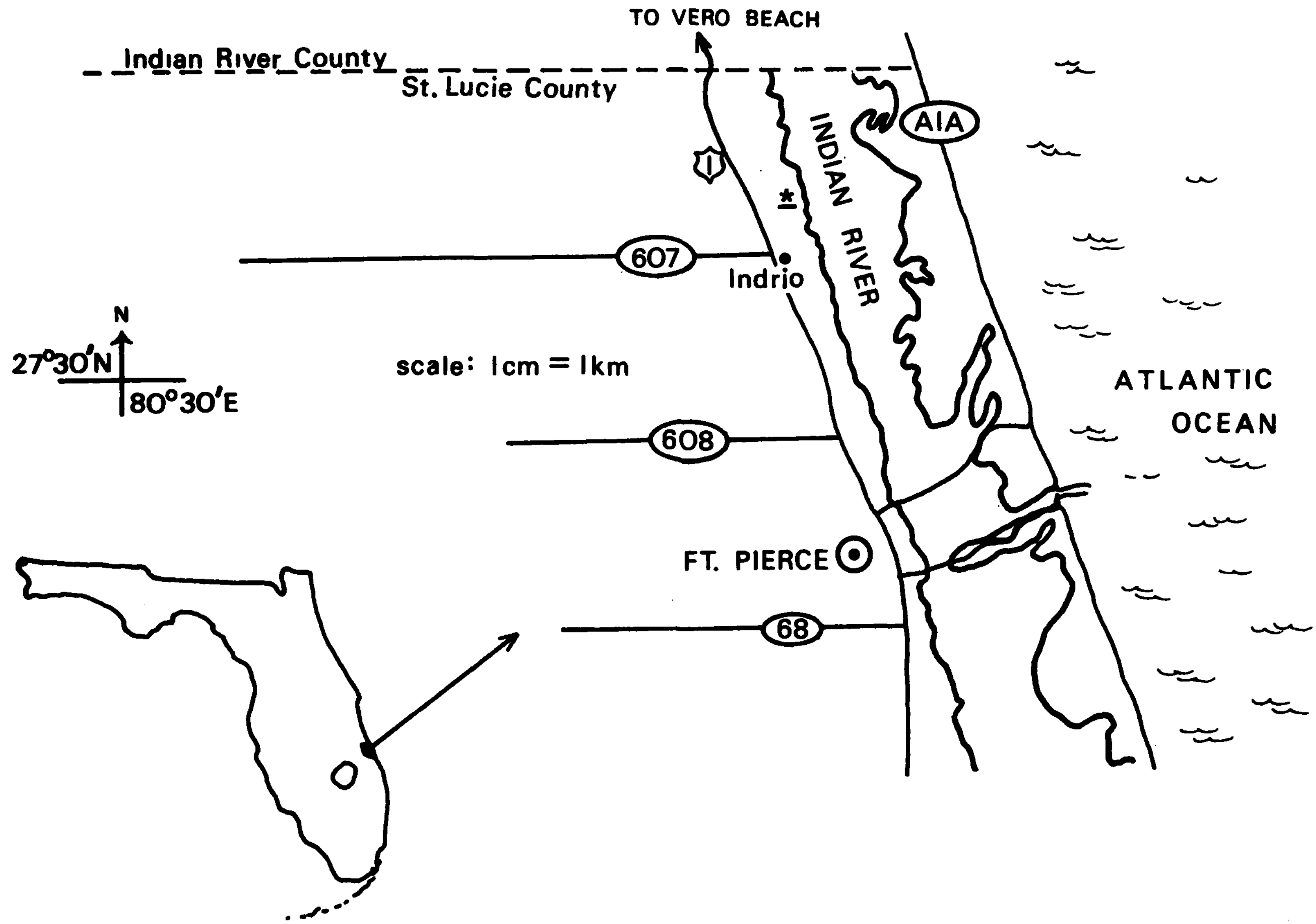



Fig. 11. Map of NE St. Lucie County, Florida indicating the location (asterisk) of the canal containing Pistia stratiotes (water lettuce) and used as a study site for Mansonia mosquitoes.

than plants occurring in the floating mat at Mulberry. Although C. perturbans was the least abundant Mansonia recovered (Table 3), the information on closely related species is broadly applicable to the biology and control of C. perturbans.

Table 3. The abundances of the five most common mosquito species collected in two emergence traps at Indrio, Florida.

| | Trap #1: 8 Dec.-1980 - 2 Dec. 1981 | | Trap #2: 5 May-1981 - 2 Dec. 1981 | | |
|---|---|---|---|---|---|
| | males | females | males | females | totals |
| Mansonia dyari | 1622 | 1517 | 3484 | 3650 | 10273 |
| Mansonia titillans | 158 | 121 | 76 | 99 | 454 |
| Culex erraticus | 31 | 54 | 146 | 164 | 395 |
| Coquillettidia perturbans | 79 | 40 | 6 | 2 | 127 |
| Anopheles crucians | 16 | 24 | 11 | 22 | 73 |

## Emergence traps

An emergence trap (#1) of 4 $m^2$ was placed over P. stratiotes between 8 December 1980 and 2 December 1981. A second trap (#2) sampled from the same canal between 5 May 1981 and 2 December 1981. Both traps were repositioned at six to eight-week intervals (Figs. 12 & 13) to allow for the possible depletion of the subpopulation confined under the trap. Collection bottles were changed and adult mosquitoes tallied weekly. On one occasion in late March, a windstorm blew and hid trap #1 from view, and the collection bottle was not changed until after a two-week exposure; a weekly estimate was obtained by dividing this particular sample in half.

Mansonia dyari accounted for 89.7% of all mosquitoes identified from the two traps (Table 3). The other two species of Mansonia, M. titillans and C. perturbans, were relatively inabundant (Fig. 14; Table 3). Collectively, Mansonia mosquitoes accounted for 94.8% of all mosquitoes identified. The five commonest species further included Culex erraticus and Anopheles crucians and accounted for 98.9% of all mosquitoes identified. Larvae of these latter two species are also known for their associations with aquatic plants.

The patterns of seasonal abundance of the two commonest Mansonia were very similar (Figs. 14 & 15). Both M. dyari and M. titillans were apparently multivoltine. Narrow yet prominent peaks in emergence in March were followed by a ca three-month interlude of few adults. Two broad peaks, the first from late June through early August and the second from early September through early November, likely correspond to two, overlapping generations of these species. For M. dyari, males generally preceded females in abundance peaks

Fig. 12. Weekly numbers of M. dyari recovered from trap #1 at Indrio, Florida. Open circles represent males, closed circles, females; arrows denote relocations of the trap.

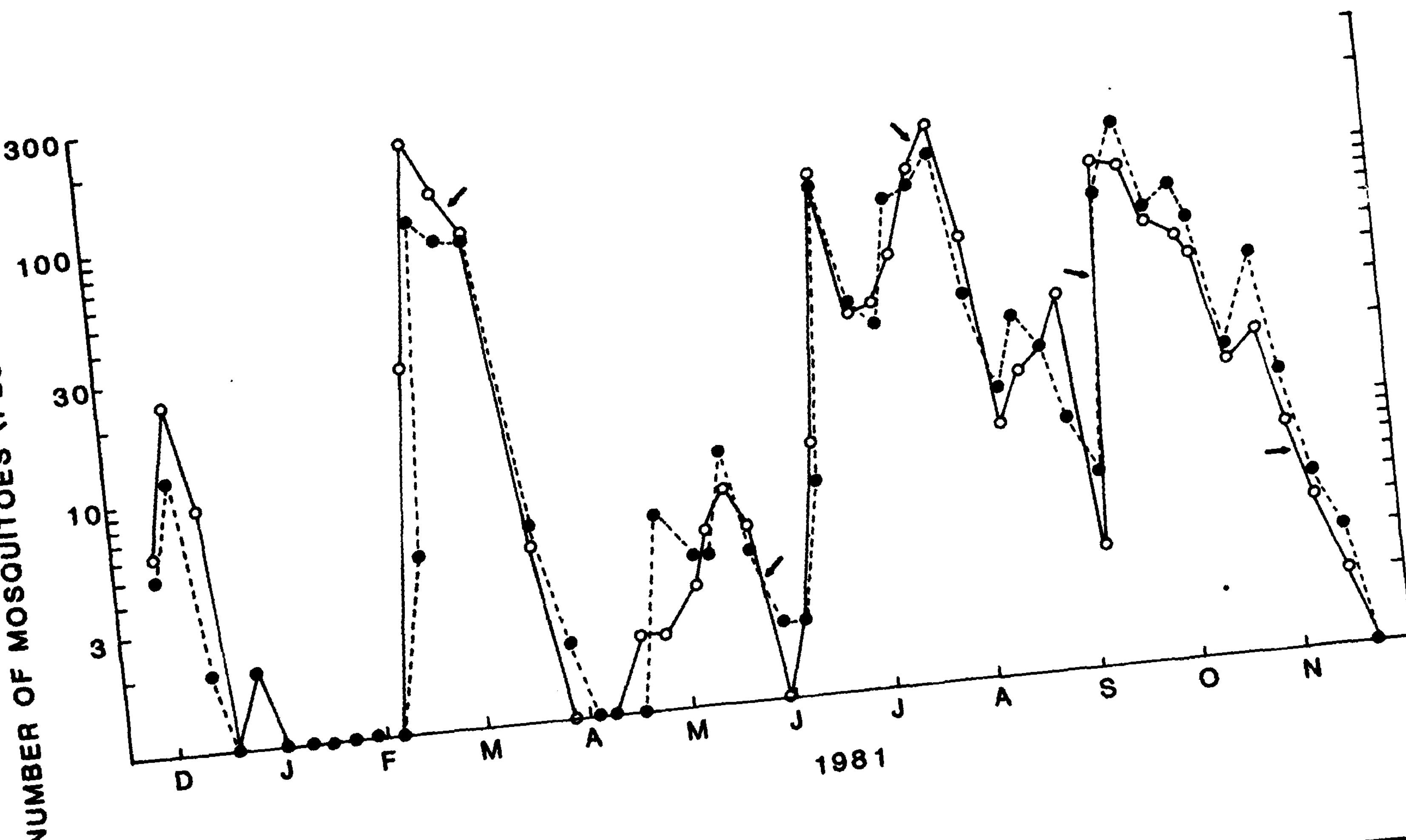


Fig. 13. Weekly numbers of M. dyari recovered from trap #2 at Indrio, Florida. Open circles represent males, closed circles, females; arrows denote relocations of the trap.

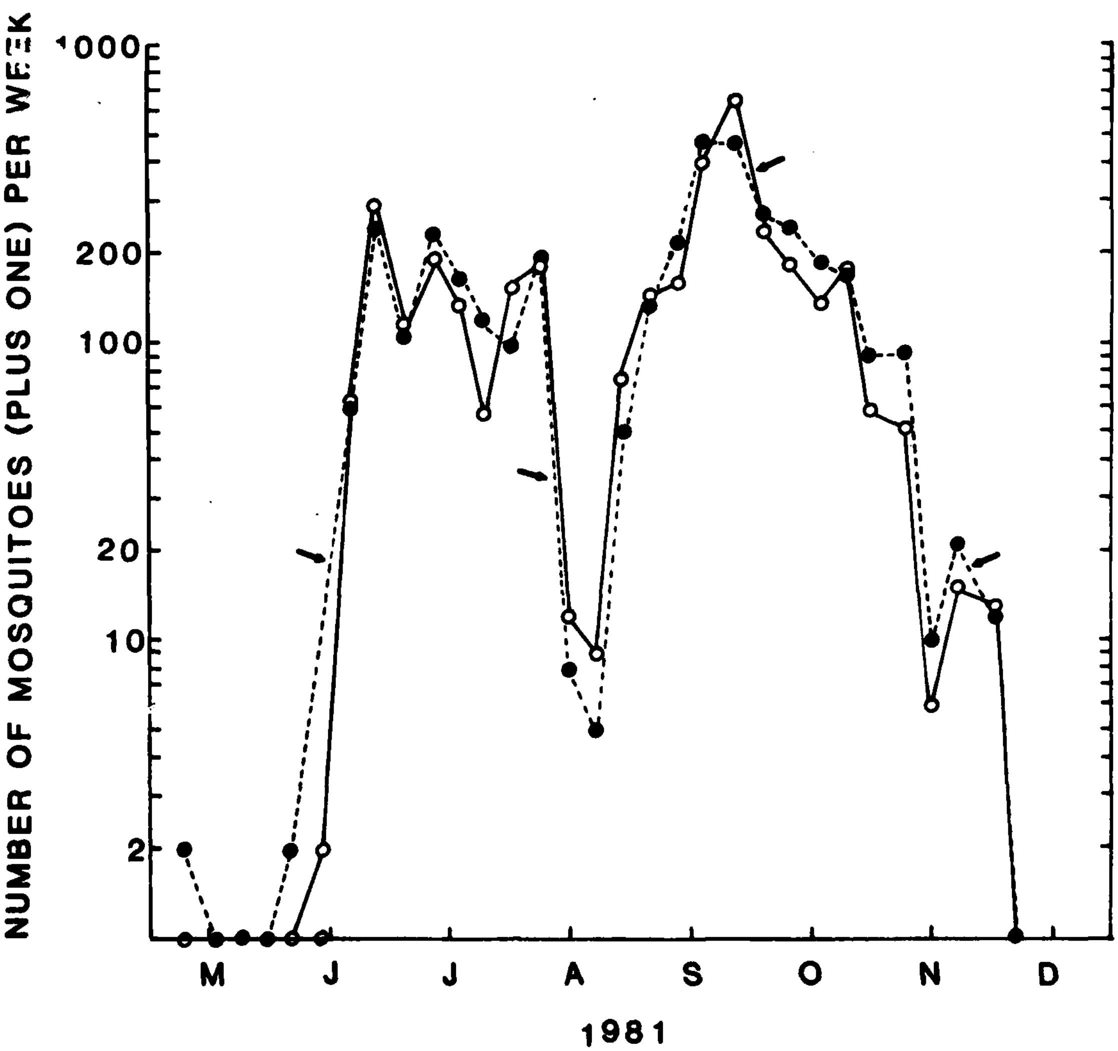


Fig. 14. Weekly numbers of <u>M. titillans</u> and <u>C. perturbans</u> captured in emergence traps at Indrio, Florida. Numbers are from one trap until May, two traps thereafter.

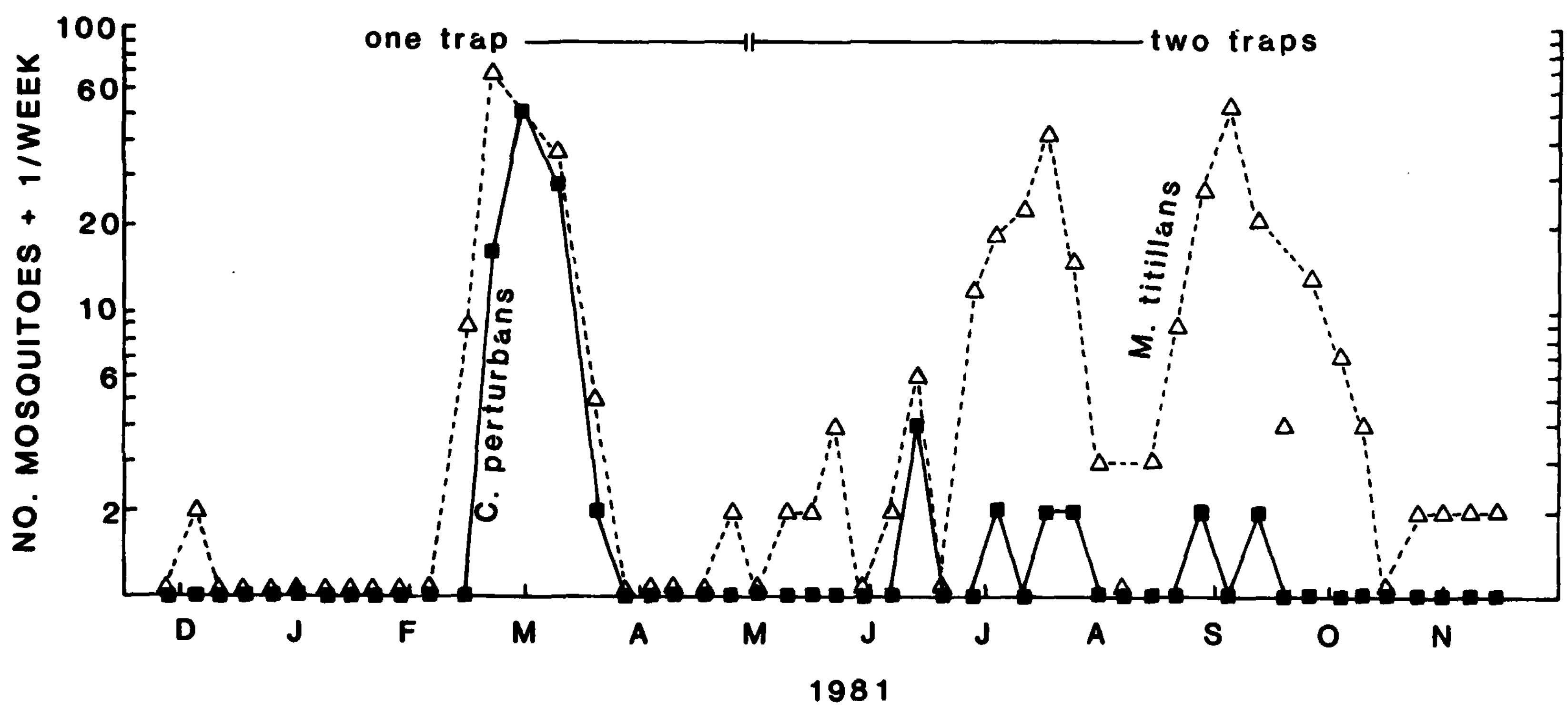
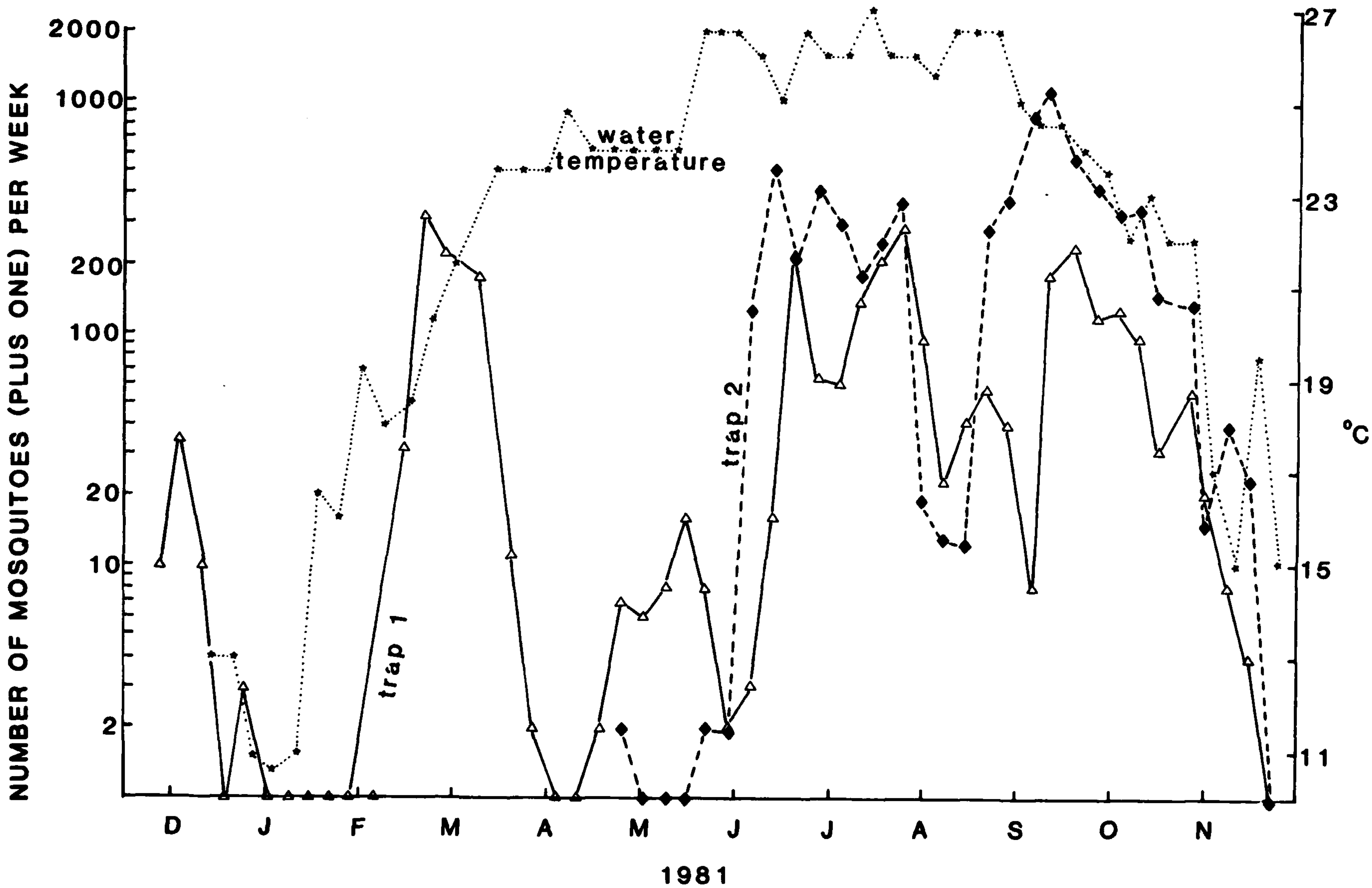


Fig. 15. Total weekly numbers of M. dyari captured in two emergence traps (4 $m^2$ each) placed on a mat of Pistia stratiotes at Indrio, Florida. Water temperatures were recorded at the level of plant roots.



(Figs. 12 & 13), supporting the interpretation that the peaks represent separate generations. Sex ratios of adults showed no overall biases, males accounting for 51.7% of M. dyari in trap #1 and 48.8% in trap #2 (Table 3).

Water temperatures at the level of Pistia roots were recorded on the occasion of removing the weekly trap collection. Temperatures ranged from 10.5°C in mid-January to a high of 27°C in late July (Fig. 15). The weekly numbers of M. dyari in trap #1 were positively correlated with water temperature recorded at the end of that week (Kendall's $\tau = 0.276$; $t_s = 2.76$, $P < 0.01$).

Only a single peak in abundance of C. perturbans was observed, coincident with the first of three surges in emergence of M. dyari and M. titillans. Thereafter, C. perturbans was rare in the two traps. Water lettuce is not regarded among the favored host plants of C. perturbans[6], and individuals constituting the lone emergence peak of this species may have entered trap #1 after detaching from other host plants, perhaps cattails which occurred along shore.

## Discussion

Based on analyses of light trap records, Provost[6] concluded that temperatures determined the abundance of M. dyari (= indubitans) in south Florida by regulating host plant abundance. Light trap data indicated two to four generations per year, peaks in abundance occurring every 90-100 days during "favorable" years. No differences in phenology between M. dyari and the rare M. titillans were observed. Bidlingmayer[6] recorded three generations of M. dyari during a year of mild temperatures in central Florida.

Data from the present study generally support these earlier observations. The emergence trap records from Indrio for 1981 suggest the occurrence of three annual generations of M. dyari and M. titillans from water lettuce. The midpoints of overlapping generations during summer and fall were separated by approximately 2.5 months. While a positive correlation between water temperatures and abundance of M. dyari was observed, there was no evidence that cold temperatures limited host plant abundance; water lettuce was common in the canal throughout the 51-week study. More likely, cold winter temperatures restrict adult emergence by retarding larval development.

Absolute numbers of mosquitoes from emergence traps at Indrio must be treated with some caution because of the presence of predators, especially adult Odonata and hylid frogs, whose impact in consuming adults within the cages is unknown. Mindful of this caveat, the maximum weekly number of M. dyari was recorded in trap #1 late in September and corresponded to an emergence rate of 280 adults/week/$m^2$ of water lettuce. Provost[5] noted from light trap data that the autumnal generation of M. dyari was the largest during most years.

## IV. Laboratory Studies on Mansonia

### Culture techniques for larval C. perturbans

The success of Guille[7,8] in rearing the European Coquillettidia richiardii on wheat stems cultured hydroponically, prompted us to use this plant for larval attachment of C. perturbans in the laboratory. Wheat seeds were germinated in a commercial sprouter, and roots for attachment maintained in liter jars half-filled with distilled water. Although wheat roots apparently provided a satisfactory substrate, larval development was exceedingly slow. The shortest developmental time from egg hatch to pupation was approximately three months, and total mortality was high (Table 4). Even when weekly mortality averaged only 5 - 10%, only 10-12% of first instar lavae survived to the pupal stage.

Table 4. Examples of cultures of C. perturbans which produced some pupae on wheat roots mainained at 26 ± 1°C in liter jars with distilled water. Photoperiods were 14L:10D except for the cohort of 34 I which was maintained at 10L:14D.

| Original Cohort | Source | % Pupation | Time (d) to Last pupa |
|---|---|---|---|
| 20 II* | field-collected from cattails 13/VII/78 | 30 | 45 |
| 13 IV | field-collected from cattails 27/VII/78 | 62 | 29 |
| 34 I | from eggs hatched 23/X/80 | 12 | 152 |
| 51 I | from eggs hatched 6/VI/81 | 10 | 105 |
| 49 I | from eggs hatched 6/VI/81 | 12 | 96 |
| 90 I | from eggs hatched 23/VI/81 | 10 | 90 |

*Roman numerals refer to instar number

Various modifications of the culture technique provided no consistent improvement in the percentages of C. perturbans completing development. Alterations included (1) the addition of food: protozoan infusions[9], powdered yeast and/or lactalbumin; (2) the use of alternative attachment substrates: various papers[10], styrofoam, or roots of other plants; (3) gentle aeration of the cultures; (4) lowering of rearing temperatures to 15° or 20°C. After many trials and failures we concur with the frustration of Laurence et al. who concluded: "... generally it has been impossible to associate poor larval culture with any similar change in the larval infusions."[10]

diapause

Since the culture of larvae proved unreliable, experiments on defined photoperiods, temperatures, and food led to no conclusions concerning the relative importance of these factors in diapause induction, maintenance, or termination. However, some data from laboratory cultures suggested a change in the physiological status of the overwintering larval population. Fourth-instar larvae field-collected from Mulberry between November and April were placed on wheat roots and exposed to various photoperiods, temperatures, and food conditions. When the data from all cultures were pooled, it appeared that larvae collected in the spring were more likely to pupate in culture than larvae collected in the autumn or winter (Table 5). These data suggest that the larval dormancy of C. perturbans is a bona fide diapause that requires a hibernal activation period before the insect is maximally sensitive to stimuli promoting development.

Table 5. The incidence of pupation among cultures of field-collected fourth instar C. perturbans.

| Collection Date | Number of IV Larvae | Cultures producing pupae/total no. cultures | % with pupae |
|---|---|---|---|
| 5/XI/79 + 6/XII/79 | 552 | 2 / 10 | 20 |
| 30/I/80 | 213 | 4 / 13 | 31 |
| 27/II/80 | 296 | 4 / 20 | 20 |
| 26/III/80 | 640 | 19 / 32 | 59 |
| 23/IV/80 | 1280 | 24 / 29 | 83 |

attachment behavior

The larval sampling program, which required pulling host plants from their aquatic medium, raised the question of how many Mansonia larvae were being dislodged during the uprooting of plants. Two laboratory experiments were established to determine the reaction of Mansonia to disturbances that might provoke detachment from their host plants. In the first of these, known numbers of C. perturbans larvae were allowed 24 hr in a beaker with 500 ml of water to attach to wheat roots. The beaker further contained a magnetic stir bar which could be controlled to produce a quantifiable and repeatable disturbance (a vortex) in the medium. After several trials, a setting on the stir plate was determined which usually detached at least some larvae. The vortex required to dislodge larvae was considerable, achieved only near the maximal turning speed of the stir bar. At this setting, the percentages of larvae detaching did not differ among instars (IV: 8/19 = 42%; III: 11/20 = 55%; I: 2/4 = 50%).

The second experiment simulated more closely the disturbance experienced by larvae when roots are pulled from their aquatic substrate. Known numbers of larvae of C. perturbans or M. dyari were allowed to attach at three temperatures to wheat roots in beakers. The roots were then lifted by a gentle and even pull on their stems and transferred to an adjacent beaker. The number of larvae which were successfully transferred on the roots were then counted. Results indicated differences in attachment between M. dyari and C. perturbans (Table 6); the former species proved far more tenacious in its attachment behavior. Conceivably, this behavioral difference is related to the different types of plants favored for attachment. Floating plants, such as P. stratiotes, favored by M. dyari are probably more subject to disturbance by wind and animals, and it may be adaptive for M. dyari larvae to be firmly secured in the event of such agitation. Of further interest are the data in Table 6 which indicate that the greatest percentage of C. perturbans were transferred at the lowest temperature tested. These results are pertinent to the field data of Fig. 6 which showed that more larvae were recovered per cattail at lower temperatures. Collectively, these data support the hypothesis that the trend of increased larval densities in the autumn and winter are sampling "artifacts" caused by improved recovery of samples in colder weather.

Table 6. The transfer on wheat roots of Mansonia larvae between two laboratory beakers.

| | Water Temp. (°C) | | No. Trials | No. Attached | No. Transferred | % |
|---|---|---|---|---|---|---|
| C. perturbans III + IV | 16.5 | | 4 | 23 | 15 | 65.2 |
| | 20.0 | | 10 | 34 | 16 | 47.1 |
| | 24.0 | | 4 | 29 | 14 | 48.3 |
| | | Totals | 18 | 86 | 45 | 52.3 |
| M. dyari III + IV | 16.5 | | 8 | 56 | 55 | 98.2 |
| | 20.0 | | 15 | 45 | 42 | 93.3 |
| | 24.0 | | 8 | 40 | 38 | 95.0 |
| | | Totals | 31 | 141 | 135 | 95.7 |

## V. Conclusions and Recommendations

This study has provided biological information which may be applied to the control of C. perturbans in particular and Mansonia mosquitoes in general. Relevant results and their applicability include the following. Populations of C. perturbans overwinter exclusively in their larval stages and, therefore,

larvicides may be most profitably applied between November and March. The first emergence of *C. perturbans* adults is highly predictable. Early during spring emergence sex ratios are male-biased, such that adulticiding may be delayed until females predominate. As cattail size and number of larvae are positively correlated, larger plants may be expected to produce greater infestations of *C. perturbans*. To assess *Mansonia* abundances for purposes of local control, larval estimates are best made in the winter months when plant-insect attachment is firmest, and adult abundances may be sensitively monitored year-round by the use of emergence traps. The correlations reported here may lead to predictive relationships between water temperatures and abundances of *C. perturbans* larvae and adults.

Further field studies of *C. perturbans* and *Mansonia* relatives are needed in the diverse habitats in which they occur in south Florida. Particularly valuable sequels to the current investigation would include the development of additional, creative sampling techniques and the experimental manipulation of confined *Mansonia* habitats towards the goal of reducing mosquito production. To evaluate certain aspects of their life history, *Mansonia* species must be colonized in the laboratory. Finally, while the present study has focused on the biology of the mosquito, human land use practices are culpable for the major *Mansonia* infestations in south Florida. The most expedient solutions to these mosquito infestations involve repair of those habitats damaged by man.

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## VII. Publications and Personnel

No publications have been made with the support of the present contract.

The following individuals received support from the contract:

| | |
|---|---|
| L. P. Lounibos, Entomologist III (cost-sharing with State of Florida) | July 1979 - October 1981 |
| Richard L. Escher, Entomologist II | October 1979 - October 1981 |
| Cindy L. Vandover, Technician (OPS) | June 1980 - October 1981 |
| Linda J. Moore, Entomologist II (temporary) | July 1979 - October 1979 |

## VIII. Distribution List

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